CRYSTALLINE PEPSIN

VI. Inactivation by Beta and Gamma Rays from Radium and by Ultra-Violet Light

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Previous work (1) has shown that when solutions of crystalline pepsin are inactivated by alkali or by heat the loss in activity is exactly proportional to the loss of native protein. These experiments confirm the idea, therefore, that the native protein molecule is the active enzyme. Proteins are denatured (2) by exposure to radium or to ultra-violet light and it is also known that pepsin solutions (3) are inactivated under these conditions. Inactivation of the enzyme by radium or ultra-violet light, therefore, furnishes another method of testing the relationship between the protein and the active molecule. If the protein molecule itself is responsible for the activity then any loss in activity must be accompanied by a corresponding decrease in the protein. On the other hand, if a hypothetical, active molecule is merely associated with the native protein there is no reason to suppose that the rate of inactivation of the active molecule would be the same as the rate of denaturation of the protein. The inactivation of pepsin solutions has been studied from this point of view and it has been found that the loss in activity is just proportional to the loss in native protein when the enzyme is inactivated either by radium or by ultra-violet light. These results, therefore, furnish additional evidence in favor of the idea that the protein molecule itself is responsible for the activity.

EXPERIMENTAL RESULTS

I. Decrease in Activity of Protein Nitrogen of Pepsin Solutions Exposed to Radium Bromide at pH 5.0 and 0°C.

The results of an experiment in which pepsin solutions of various concentrations were exposed to the radiation of 100 mg. of radium

bromide at 0°C. are shown in Table I. The decrease in activity, as determined by the hemoglobin method, is just proportional to the

TABLE I

Changes in Activity and Protein Nitrogen of Pepsin Solutions Exposed to Radium Bromide, pH 5.0, 0°C.

hrs.									
	$[P.U.]^{Hb}_{ml.}$	0.5	0.2	0.043	0.048	0.010	0.010	0.0048	0.0048
0	P N/ml., *mg.*	2.6	1.0	0.24	0.22	0.050	0.050	0.023	0.023
	$[P.U.]^{Hb}_{mg.PN}$	0.19	0.20	0.21	0.22	0.20	0.20	0.21	0.21
	$[P.U.]^{Hb}_{ml.}$							0.0041	
25	P N/ml., *mg.*							0.020	
	$[P.U.]^{Hb}_{mg.PN}$							0.20	
	$[P.U.]^{Hb}_{ml.}$			0.037					0.0036
72	P N/ml., *mg.*								0.019
	$[P.U.]^{Hb}_{mg.PN}$								0.19
	$[P.U.]^{Hb}_{ml.}$				0.031		0.0054		
96	P N/ml., *mg.*				0.16		0.030		
	$[P.U.]^{Hb}_{mg.PN}$				0.19		0.18		
	$[P.U.]^{Hb}_{ml.}$	0.5		0.021		0.0056			
160	P N/ml., *mg.*	2.5		0.10		0.035			
	$[P.U.]^{Hb}_{mg.PN}$	0.20		0.21		0.15			
	$[P.U.]^{Hb}_{ml.}$		0.17						
190	P N/ml., *mg.*		0.78						
	$[P.U.]^{Hb}_{mg.PN}$		0.22						
	$[P.U.]^{Hb}_{ml.}$	0.43							
310	P N/ml., *mg.*	2.17							
	$[P.U.]^{Hb}_{mg.PN}$	0.20							
	$[P.U.]^{Hb}_{ml.}$		0.083						
770	P N/ml., *mg.*		0.48						
	$[P.U.]^{Hb}_{mg.PN}$		0.17						

decrease in the protein nitrogen of the solution as shown by the fact that the activity per mg. protein nitrogen remains constant. No denatured protein appears in the solution although it is probable

that the first step in the reaction is the formation of denatured protein. The rate of denaturation under these conditions, however, is extremely slow and the denatured protein, if present, would undoubtedly be digested by the remaining active native protein as rapidly as it was formed and so does not accumulate in the solution.

Effect of the Concentration of Pepsin

Below about 0.05 mg. nitrogen per ml. the per cent inactivated is nearly constant, while in more concentrated solutions the actual quantity inactivated is approximately constant. Similar results were obtained by Hussey and Thompson (3). They indicate that, under the conditions of the experiment most of the energy is absorbed by 0.05 mg. nitrogen per ml. so that increasing the concentration beyond this point does not have much effect upon the number of protein molecules inactivated.

Experimental Procedure

A solution of three times crystallized pepsin was prepared in pH 5.0 N/20 acetate buffer and diluted to the concentrations noted in the table with N/20 acetate buffer. 25 ml. of this solution was placed in a 1.5 cm. centrifuge tube and a glass tube (with 0.5 mm. walls) containing 100 mg. of radium bromide suspended in the center of the solution. The tube was kept in the ice box at 0°C. and 1 ml. samples removed and analyzed for protein nitrogen and activity as noted in the table.

Activity Determinations.—The activity determinations were made with hemoglobin by the method of Anson and Mirsky(4).

Protein Nitrogen Determination.—1 ml. of solution added to 5 ml. of 5 per cent boiling trichloracetic acid, the precipitate centrifuged and washed three times with 5 per cent trichloracetic acid, and total nitrogen in the precipitate determined.

Test for Denatured Protein.—1 ml. of solution added to 10 ml. of N/2 sodium sulfate and N/20 sulfuric acid. Any denatured protein precipitates under these conditions and some of the samples which had been almost completely inactivated gave a slight cloud. The amount of denatured protein was, however, too small to determine.

II. Inactivation by Ultra-Violet Light

A. Changes in Protein Nitrogen and Activity of Pepsin Solutions at Various pH Exposed to Ultra-Violet Light

The results of an experiment in which pepsin solutions at various pH were exposed to light from a mercury arc are shown in Table II. As in the case of radium inactivation practically no denatured protein

TABLE II

Change in Activity and Protein Nitrogen in Pepsin Solutions of Various pH Exposed to Ultra-Violet Light

pH.....	0			1.7			3.0			4.65		
Buffer.....	1.0 N hydrochloric acid			N/50 hydrochloric acid			N/65 acetic acid			N/50 4.65 acetate		
Time	$[P.U.]^{Hb}_{ml.}$	P N/ml.	$[P.U.]_{mg.\ P\ N}$	$[P.U.]^{Hb}_{ml.}$	P N/ml.	$[P.U.]_{mg.\ P\ N}$	$[P.U.]^{Hb}_{ml.}$	P N/ml.	$[P.U.]_{mg.\ P\ N}$	$[P.U.]^{Hb}_{ml.}$	P N/ml.	$[P.U.]_{mg.\ P\ N}$
hrs.		*mg.*			*mg.*			*mg.*			*mg.*	
0	0.019	0.098	0.20	0.020	0.098	0.21	0.020	0.098	0.22	0.020	0.098	0.21
2.55	0.014	0.076	0.18	0.015	0.070	0.215	0.017	0.080	0.21	0.017	0.084	0.20
5.00	0.0092	0.058	0.16	0.0095	0.050	0.19	0.0118	0.070	0.17	0.013	0.078	0.17
8.75	0.0054	0.030	0.18	0.0050	0.018	0.28	0.0077	0.058	0.13	0.0086	0.063	0.14

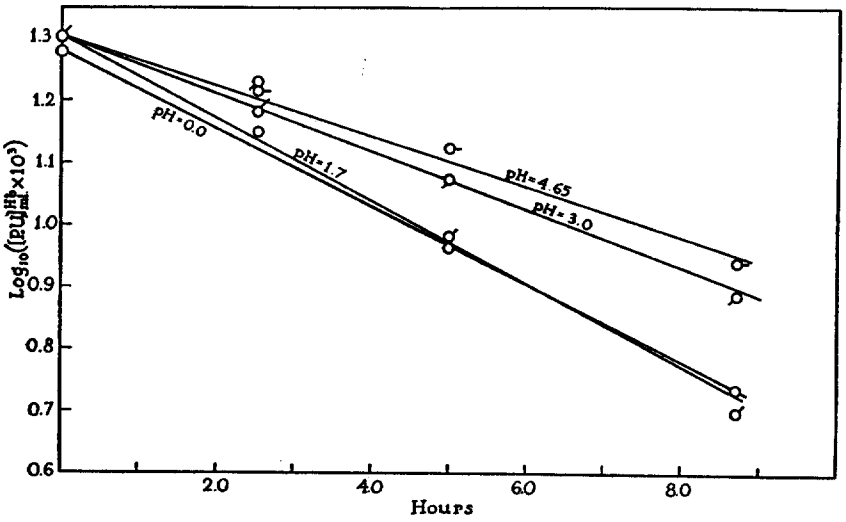

FIG. 1. Inactivation of pepsin by ultra-violet light at different pH

appears in the solution and the loss in activity is accompanied by a corresponding decrease in the total protein nitrogen of the solution; *i.e.*, the activity per mg. protein nitrogen remains constant throughout the experiment. The rate of inactivation depends upon the pH and is a maximum at about pH 2.0 and decreases as the pH becomes more

alkaline. The pH corresponding to the maximum rate of inactivation agrees with that found by Collier and Wasteneys (5) and is slightly less acid than that reported by Pincussen and Vehara (3). The reaction follows approximately a monomolecular course, as shown in Fig. 1, in which the log of the activity is plotted against the time in hours.

Experimental Procedure

A solution of three times crystallized pepsin was prepared in N/20 pH 4.65 acetate. It contained 2.5 mg. protein nitrogen per ml. 1 ml. of this solution was diluted with 25 ml. of the buffer noted in the table and the pH determined. 25 ml. of the solution was placed in 1 cm. quartz test-tubes arranged in a semi-circle around a General Electric "Lab-Arc" at a distance of 8.5 cm. from the arc. The lamp was operated on 110 volts A.C. and 1.8 amperes and was allowed to run 1 hour before the experiment was started. The activity and protein nitrogen were determined, as described for the radium experiments, except that with very dilute pepsin solutions it was necessary to use 5 ml. for the protein nitrogen determination. Control tubes containing 25 ml. of the solution in glass test-tubes were placed beside the quartz tubes. There was no change in activity or protein nitrogen in the solution in the glass tubes showing that the inactivation was due entirely to ultra-violet light. The temperature of the solutions was 15°C. The positions of the quartz tubes were interchanged at intervals of about 20 minutes so that any local variations in the light intensity were distributed. Special control experiments showed that the rate of inactivation was the same in the various tubes.

The analytical work was done by Mr. Nicholas Wuest.

SUMMARY

1. The loss in activity of crystalline pepsin solutions when exposed to beta and gamma rays from radium or to ultra-violet light is accompanied by a corresponding decrease in pepsin protein.

2. The rate of inactivation by ultra-violet light depends upon the pH and is a maximum at about pH 2.0.

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